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THE DEMONSTRATION OF LYSOSOMES IN THE DISEASED SKIN OF INFANTS WITH INFANTILE ECZEMA*

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In a recent report (1), the ultrastructure of the epidermis in the exudative infantile eczematous skin was described. In the zone immediately subjacent to the parakeratotic stratum corneum, many keratinocytes contained moderate numbers of granules with distinctive morphologic features; they were round or ovoid, moderately electron dense, 0.1 to 1.0 mμ in diameter, and sharply delimited from the surrounding cytoplasm. Since these structures were seen in close proximity to tonofilaments and, in the grids examined, they were not membrane bound, it was thought that they represented aberrant keratohyalin granules. Further studies of these lesions, to be reported herein, have shown that these granules are lysosomes. Many cytolysosomes or autophagic vacuoles were also identified within these cells.

MATERIALS AND METHODS

From each of six patients, 3 months to 1 year old, a specimen of diseased skin, 0.3 cm in diameter, was removed with an electrically driven punch from the upper or lower extremity. For routine electron microscopy, the specimens were cut into 1 mm fragments, fixed in phosphate buffered 3% glutaraldehyde, post-fixed in phosphate buffered 1% osmium tetroxide, passed through graded alcohols, and embedded in Epon. When acid phosphatase and electron microscopic studies were combined, the specimens of skin were fixed in 4% formaldehyde containing 1% calcium chloride and 5% sucrose at 4°C for 18 hours. Sections, up to 50 μ in thickness, were cut on a freezing microtome. They were incubated in a modified Gomori's medium (2) at 37°C for 5 to 15 minutes, post-fixed in phosphate buffered 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. Thin sections were cut with glass or diamond knives on a Porter-Blum microtome, stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I. The sections were magnified up to 20,000 diameters and photographically enlarged as desired.

RESULTS

The keratinocytes situated just below the stratum corneum were usually larger than those seen in the same zone of healthy skin. Nevertheless, their long axes were parallel to the skin surface. Most of these cells contained a single, large ovoid or kidney-shaped nucleus, with one or more nucleoli, and focally clumped karyoplasm. A "clear" zone, free of tonofilaments, surrounded the nucleus (Fig. 1). These cells appeared to contain more than their normal complement of cytoplasmic structures (Fig. 2), *i.e.* mitochondria, vesicles (some surrounded by rough-surfaced endoplasmic reticulum), small dark particles (presumably ribonucleoprotein, RNP), glycogen, lipid granules, melanin granules, and lamellated membrane-coating particles (3). The tonofilaments were grouped together, but tended to remain distinct within these aggregates (Figs. 1, 2), and measured approximately 50 Å in width. They were attached to the outer cytoplasmic membrane adjacent to desmosomes which were normal in appearance. Occasional keratinocytes had a few, small, dense keratohyalin granules within strands of aggregated tonofilaments. The intercytoplasmic spaces, containing fragments of membrane-coating particles, were considerably dilated and filled with amorphous material of moderate electron density.

In addition to the usual structures listed above, lysosomes were present within the keratinocytes comprising the stratum granulosum in 3 of the 6 specimens of skin examined. Although pleomorphic, these single membrane bound structures were frequently round or ovoid, approximately 0.1 to 1.5 μ in diameter, and tended to be most numerous in the perinuclear region (Figs. 1, 2). Indeed several of these lysosomal granules were clearly in proximity to a hypertrophied Golgi apparatus (Fig. 3). They ranged in appearance from light and mottled (of low electron density) to uniformly dark grey (Figs. 1, 2). Their internal

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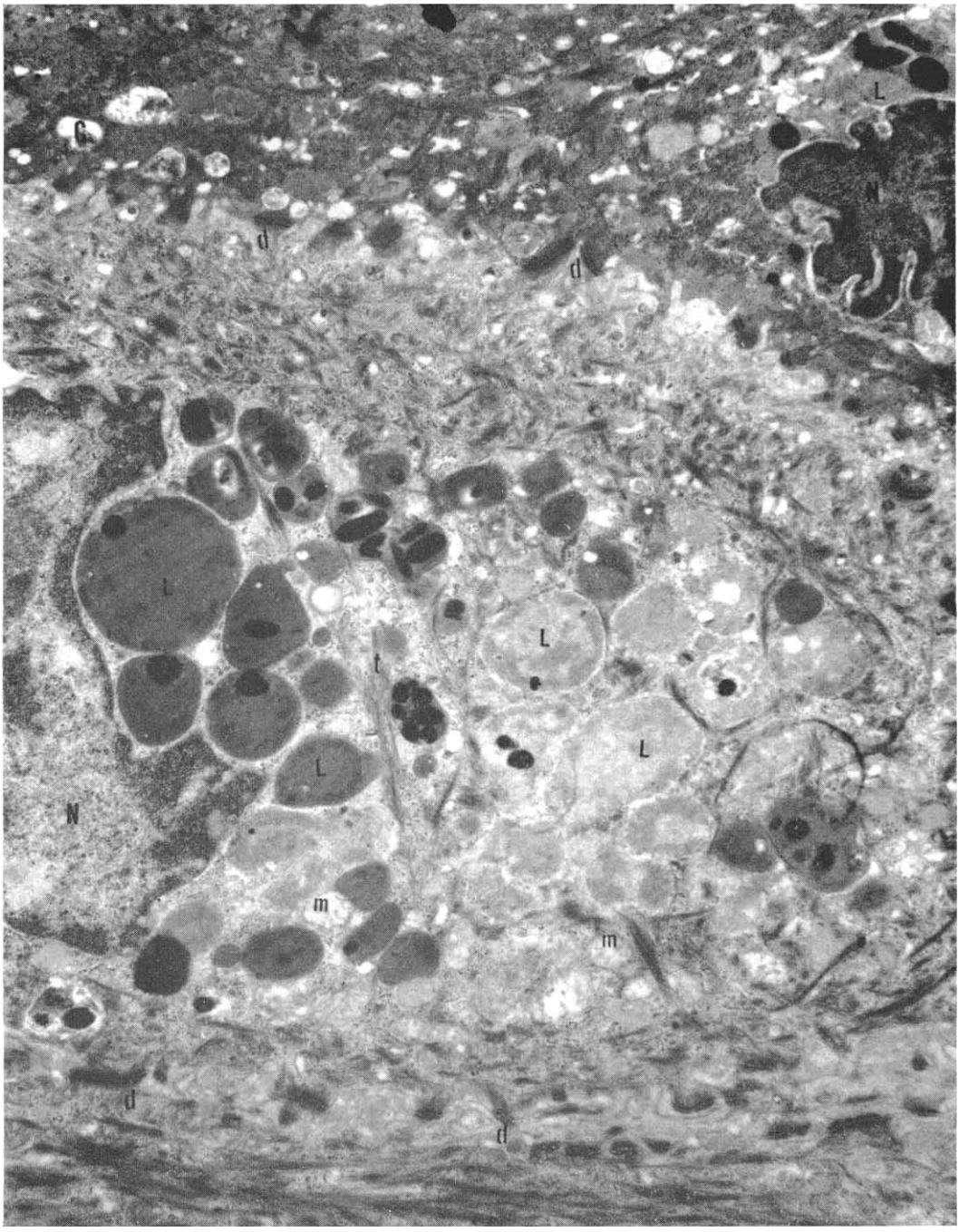


Key to abbreviations used in figures 1 to 9.

C = stratum corneum	L = lysosome or cytolysosome
d = desmosome	li = lipid granule
G = Golgi apparatus	M = multivesicular body
g = granule	m = mitochondrion
gly = glycogen	r = small dark granules (ribosomes?)
is = intercellular space	t = tonofilaments

FIG. 1. Parakeratotic stratum corneum (upper part of the electron micrograph) and large keratinocyte in the subjacent stratum granulosum are shown. This keratinocyte does not contain keratohyalin granules. The central and mid-zonal regions of the cytoplasm are largely occupied by varied sized lysosomes which range in appearance from a mottled light grey to a dark grey. Several of these structures possess round or ellipsoid, black melanin granules. Within the stratum corneum, lysosomes and cytolysosomes are shown in the perinuclear region of a nucleated keratinizing cell (right upper part of the electron micrograph). × 15,000

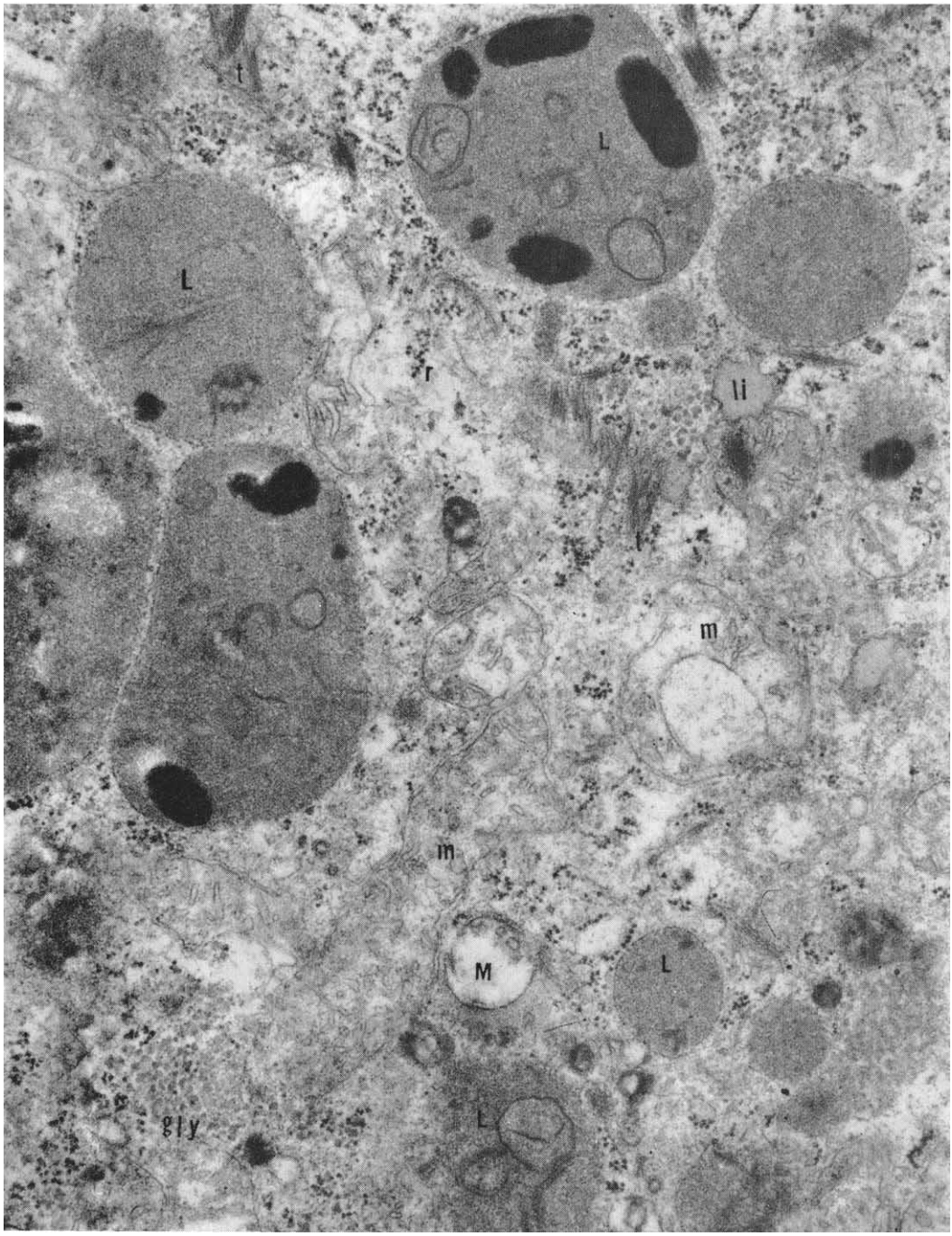


FIG. 2. Cytoplasm of a keratinocyte in the stratum granulosum showing single membrane bound lysosomes, cytolysosomes which contain spiralled membranes and/or melanin granules, and a multivesicular body. × 38,500

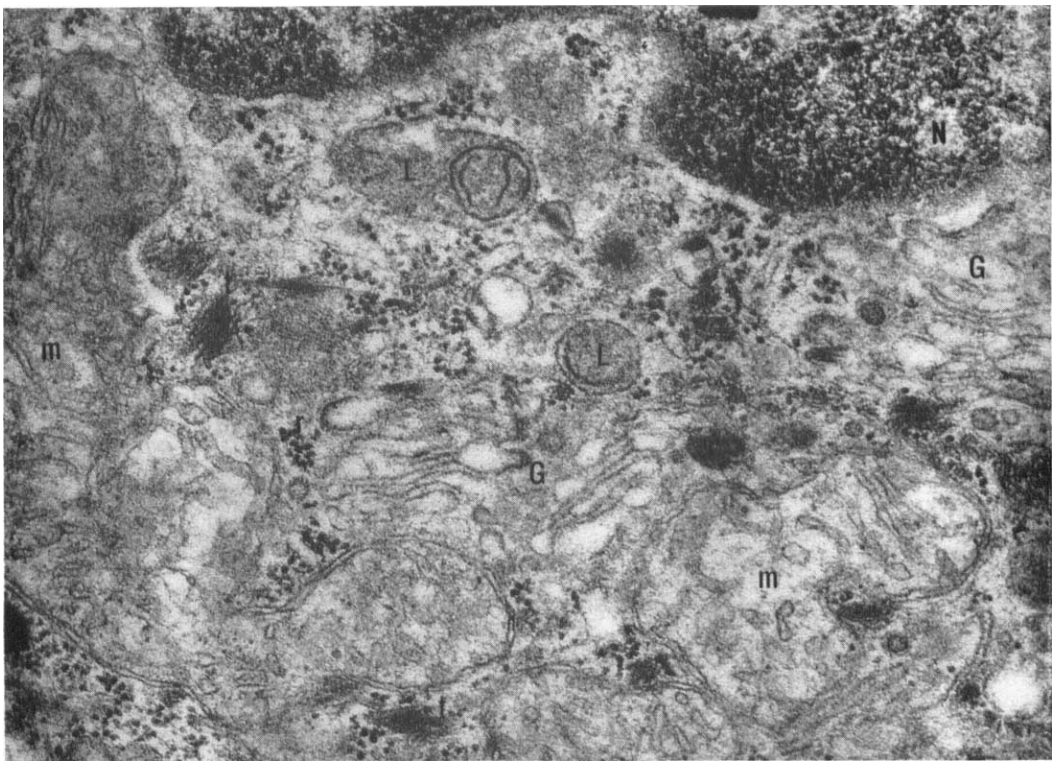


FIG. 3. Perinuclear zone of a keratinocyte in the stratum granulosum showing hypertrophied Golgi apparatus and small cytolysosomes containing spiralled membranes. × 54,000

structure could not be clearly resolved: some appeared amorphous; others, granular; and a few seemed to contain fine tubules. Some granules contained degenerating cytoplasmic components and were morphologically consistent with cytolysosomes or autophagic vacuoles. Among these components were melanin granules (Figs. 1, 2, 4), mitochondria, small dark particles, lamellated membrane-coating particles, or spiralled membranes in various stages of degeneration (Figs. 2, 3). Some cytolysosomes contained a melange of these cytoplasmic structures (Fig. 2). Rare multivesicular bodies were found in the vicinity of lysosomes (Fig. 2).

Acid phosphatase reaction products, *i.e.* lead phosphate deposits, were found within some keratinocytes in the stratum granulosum of infantile eczematous skin. These products were usually localized in lysosomes (Fig. 5) and cytolysosomes (Fig. 6). They were seen in specimens of skin incubated with substrate for as short a time as 15 minutes, but were not found in skin incubated for 10 minutes or less.

In addition to keratinocytes, the upper epidermis contained large Langerhans' type melanocytes, some with centrioles, ordinary melanocytes, and eosinophiles. Some of these cells showed degenerative changes.

The transition to stratum corneum was abrupt (Fig. 1). The cells in this layer were irregular in size, but were usually larger than those in similar areas of healthy stratum corneum. The normal keratin pattern was not seen in these cells; instead the cells appeared to be filled with electron dense amorphous material (Fig. 7). Occasionally denser aggregates of tonofibrils coursed this amorphous material (Fig. 8). In addition, some cells possessed pyknotic nuclei, clusters of small dark particles, negative images of mitochondria and cytomembranes, melanin granules, and small, pale, round or oval, sharply circum-

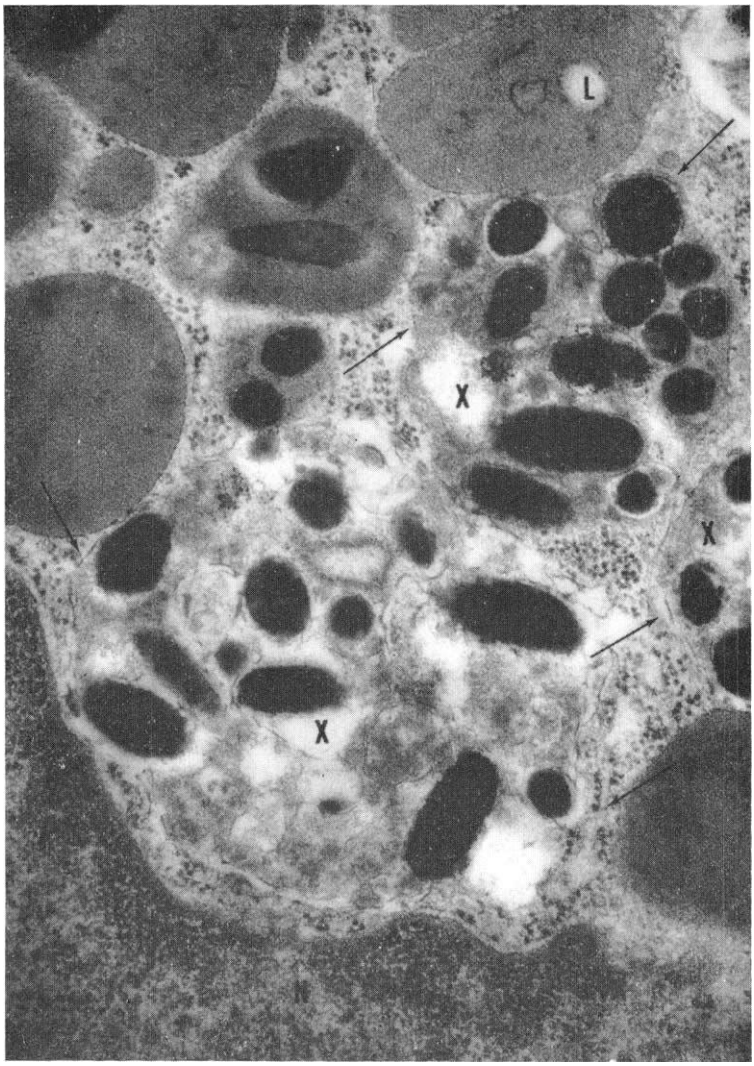
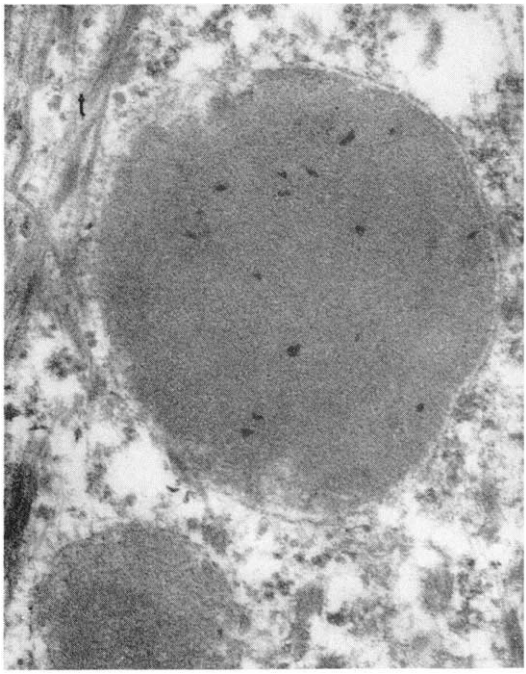


FIG. 4. Perinuclear zone of a keratinocyte in the stratum granulosum showing single membrane bound, dense lysosomes and cytolysosomes. Two large cytolysosomes (X) and part of a third, each of which is bound by a single membrane (arrows), contain melanin granules and membranes. Some of the melanin granules within these cytolysosomes are encircled by individual membranes. × 47,000

scribed granules suggestive of lipid. In the basal region of the stratum corneum, some cells showed varied sized, pale, single membrane bound lysosomes and cytolysosomes (Figs. 7, 8). The latter two structures usually appeared well preserved and were morphologically similar to those in the stratum granulosum. The cells of the stratum corneum had thickened outer cytoplasmic membranes (Fig. 7). These membranes were irregular in outline, with varied sized indentations which communicated with the intercellular spaces (Fig. 9). The desmosomes were similar in appearance to the structured desmosomes found in the lower epidermis (Fig. 7). The intercellular spaces were dilated to varying degrees (Figs. 7, 9); the widest, surrounded by thin remnants of cells, were seen in the upper stratum corneum. The spaces were filled with moderately dense amorphous material, shreds of fibrin with 220 Å cross banding, melanin and rare lipid granules.



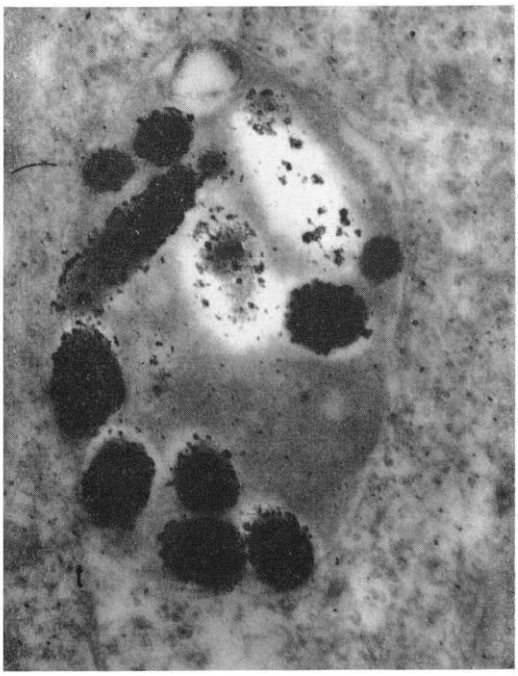


FIG. 5. Cytoplasm of a keratinocyte in the stratum granulosum showing two lysosomes. The larger one contains scattered very dark granules representing the reaction products of acid phosphatase activity after 15 minutes incubation with substrate. × 32,500

FIG. 6. Cytoplasm of a keratinocyte in the stratum granulosum showing a cytolysosome containing melanin granules, membranes, and the reaction products of acid phosphatase activity. × 51,000

DISCUSSION

The lysosome concept was originated by de Duve (4) on the basis of biochemical analyses of subcellular particles sedimenting between the mitochondrial and microsomal fractions. These particles contain many hydrolases, with optima in acid pH, capable of hydrolyzing various classes of chemical compounds. Within cells, these particles appear to function in the digestive process, and therefore, directly or indirectly, they are involved in pinocytosis, phagocytosis, and the physiologic autolysis of "worn out" or damaged cell constituents (5). Rupture of lysosomal granules would presumably result in the cell digesting itself. Lysosomes are recognized by the cytologist as pleomorphic, single membrane bound vacuoles containing electron dense material (dense bodies) (6), or cytoplasmic constituents (cytolysosomes or autophagic vacuoles) (7, 8), or small vesicles (multivesicular bodies) (9). Their identification is more definite if parallel cytochemical studies show acid phosphatase activity within these organelles.

To date, there are few published reports on epidermal lysosomes. Eisen *et al.* (10) found neither lysosomes nor their variants in keratinocytes of healthy human or psoriatic skin. Lysosomes have been observed in Langerhans' type melanocytes (11). According to Mishima (12), melanin granules can be degraded within keratinocytes. On the basis of light microscopic studies in which biochemical and routine histochemical procedures were combined, acid phosphatase activity, presumably lysosomal, is present in normal mouse skin (13). Lysosomes have been found in amphibian epidermis (10).

In view of the reports listed above, we were indeed surprised at the comparative ease with which we were able to find structures morphologically consistent with lysosomes in the keratinizing cells of the stratum granulosum and parakeratotic stratum corneum in 3 of the 6 specimens of infantile eczematous skin examined. The identification of these structures

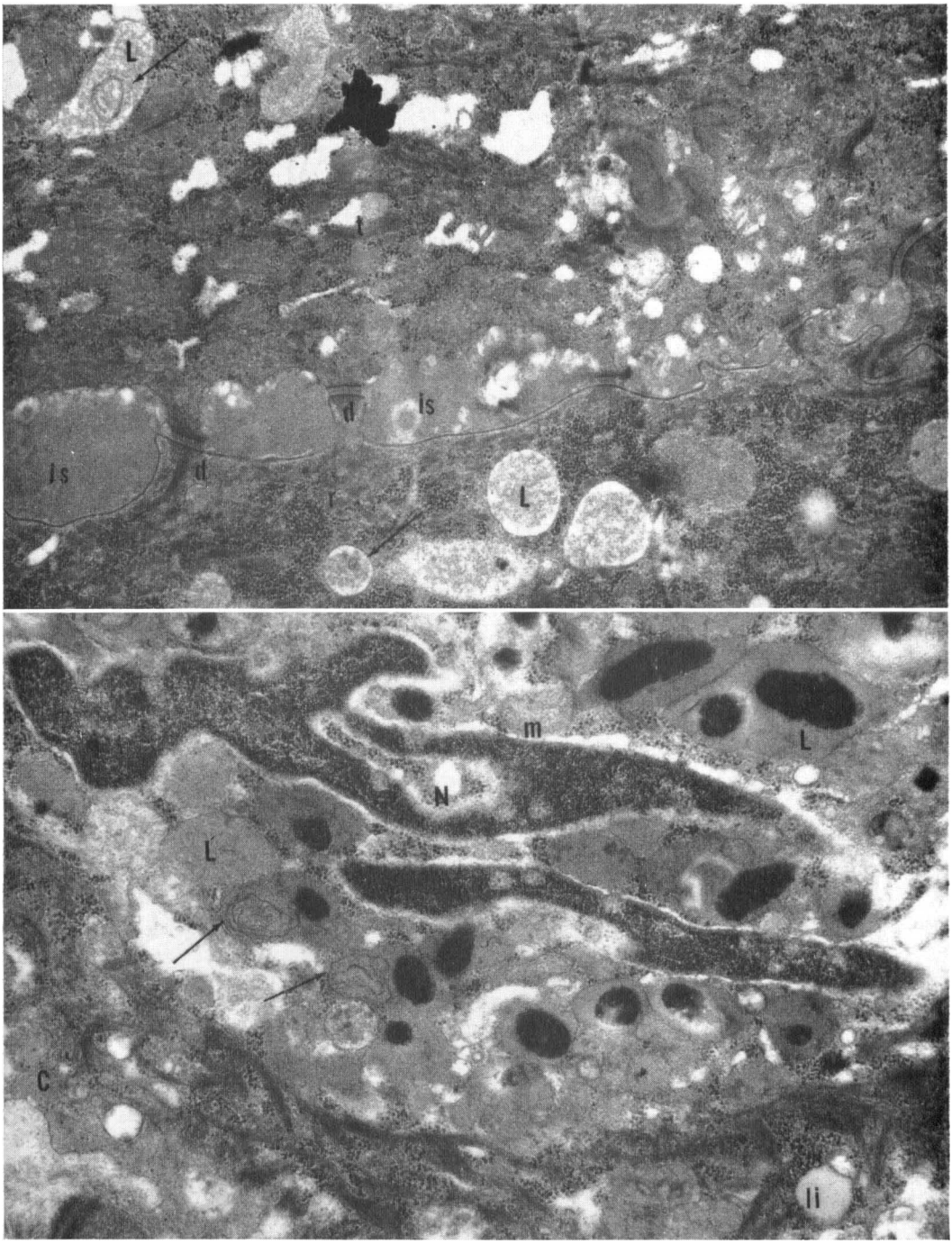


FIG. 7. Two cells in the straum corneum are shown. They do not have the normal keratin pattern. These cells contain lysosomes and cytolysosomes. The cytolysosome in the upper left part of the electron micrograph is clearly bound by a single membrane at its upper pole. Arrows indicate spiralled membranes within cytolysosomes. The outer cell membranes are dense. Structured desmosomes, similar in appearance to those seen in the lower epidermis, interrupt the dilated intercellular spaces which are filled with amorphous material. × 26,000

FIG. 8. Nucleated keratinizing cell in the stratum corneum showing cytolysosomes containing spiralled membranes (arrows) and melanin granules. A small part of a cell in the stratum granulosum is shown in the lower left corner of the electron micrograph. × 32,000

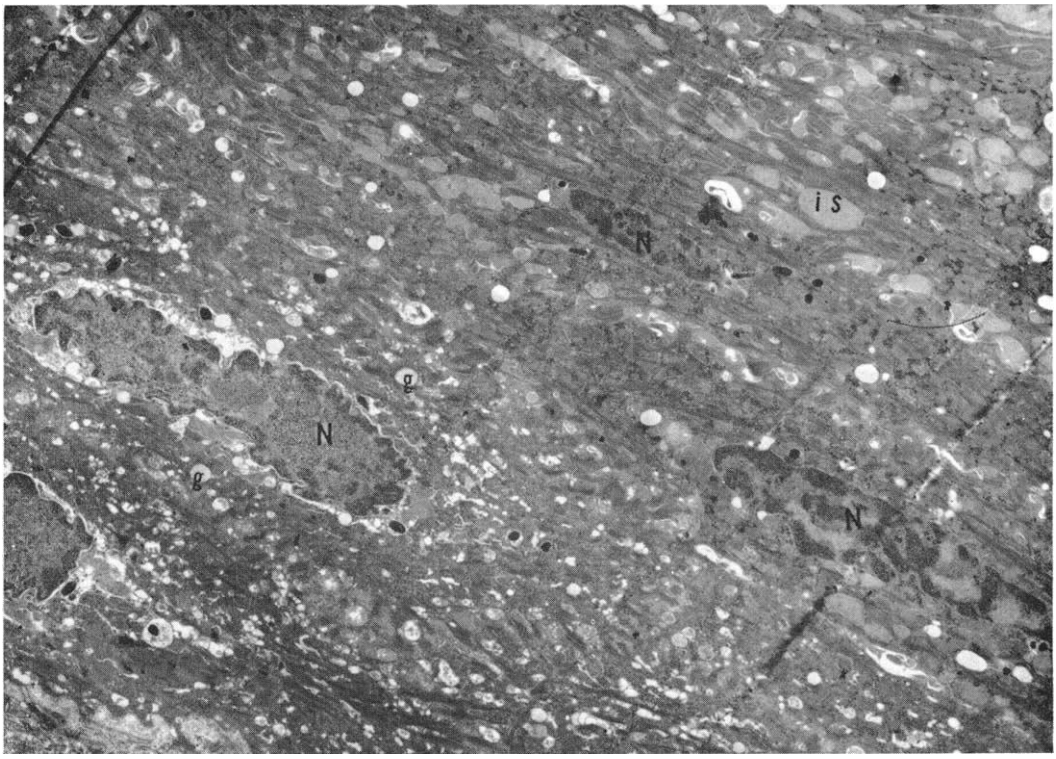


FIG. 9. Stratum granulosum (lower left corner of the micrograph) and stratum corneum are shown. The cells in the latter contain small round granules sharply delimited from the remainder of the cytoplasm. Retained nuclei are seen in some of the cells. The intercellular spaces are dilated; the dilations are more prominent in the upper regions of the stratum corneum. × 5,500

was confirmed by the observation of acid phosphatase activity within them.

Although keratinocytes in healthy and psoriatic skin may not possess lysosomes, it is now certain that under proper conditions these cells can assemble such granules. It would be of interest to try to correlate the findings in this study with the knowledge that has accumulated in regard to the formation of lysosomes in cells other than keratinocytes. The reaction products of acid phosphatase activity have been located in the Golgi apparatus and on rough surfaced endoplasmic reticulum (14). This enzyme and presumably other hydrolases are probably elaborated by RNP particles, concentrated in the Golgi apparatus, and transported by Golgi vesicles to the vacuoles destined to become lysosomes. This concept may explain the finding of hypertrophied Golgi apparati in lysosomes-containing keratinocytes and the observation of small lysosomes neighboring on the Golgi system. The source of the membrane bounding the lysosome is thought to be the Golgi apparatus (8), invaginated outer cell membranes formed during pinocytosis or phagocytosis (5), and finally, the cytoplasm in response to hydrolyzing enzymes poured around material to be digested (7). The outer cytoplasmic membrane may very well have been the source of lysosomal limiting membranes in the keratinocytes of infantile eczematous skin, since many of the phagocytosed melanin granules were membrane bound, and many well developed lysosomes contained melanin granules. That the membrane surrounding the melanin granule was probably not the only source of lysosomal limiting membrane was suggested by other electron micrographs. In some cells it appeared that aggregates of melanin granules, each granule encircled by its individual membrane, were in turn surrounded by a larger single

membrane. Furthermore, some of the lysosomes did not show melanin granules, but contained instead cytoplasmic fragments. In these instances, the lysosomal membrane may indeed have been derived from the Golgi apparatus, a cytoplasmic vacuole, or it may have been formed de novo in the cytoplasm. Other granules resembled the dense bodies seen in normal liver parenchymal cells and possessed either melanin or cytoplasmic fragments. These may represent the end stage of lysosomal granule development, with cytolysosomes as an intermediary stage, as has been suggested by some investigators (5, 7). They have also suggested that the dense body can be re-utilized in the digestive process by emptying its enzymes into another vacuole.

In this study, lysosomes were found in the stratum granulosum and corneum. In a previous study of infantile eczematous skin (1), multivesicular bodies or compound vesicles were found within keratinocytes of the basal cell layer. Since these bodies are probably lysosomal variants, it seems likely that lysosomes are present in all layers of the epidermis in this disease.

The persistence of lysosomes in the parakeratotic stratum corneum was observed in this study. This finding would seem to indicate that these granules were stable and served an autodigestive function, rather than a "suicidal" or "keratinocidal" one. The latter possibility can not be ruled out in severer examples of the disease. It can be surmised that, in the cases studied, lysosomes were produced in response to effete cell constituents, *e.g.*, mitochondria, ribosomes, etc.

Further studies of lysosomes in human skin, healthy and diseased, would appear to be indicated. The exudative lesions of infantile eczematous skin are evidently a good source of material for such studies. Qualitative analyses of the hydrolases in skin lysosomes, either by histochemical or biochemical technics, should eventually be possible and could provide us with very meaningful information. These enzymes may differ in different diseases and in different patients with the same disease. The course of a disease may in part depend on these differences, if they do indeed exist. Since we do know that the stability of lysosomes can be altered by drugs (15), it might prove fruitful to study the effect of medications on lysosomes in the skin and to correlate the findings with the clinical response of a disease.

SUMMARY

Keratinization in exudative, infantile eczematous skin differed from that observed in healthy skin. Within the stratum granulosum, the keratinocytes were larger than those seen in healthy skin and contained aggregates of tonofilaments in which the individual filaments remained distinct. Occasional keratinocytes possessed a few, small keratohyalin granules. The overlying, parakeratotic stratum corneum did not show the keratin pattern seen in healthy skin. Instead, the cells were filled with an amorphous material which contained tonofibrils, small dark particles (ribosomes?), and other cytoplasmic components.

One finding in the infantile eczematous skin was of particular interest. Moderate numbers of lysosomes were seen in keratinocytes in both the stratum granulosum and corneum in 3 of the 6 specimens of eczematous skin examined. The identification of these structures was confirmed by observing acid phosphatase activity within them. It is clear, therefore, that while healthy human skin keratinocytes may not contain lysosomes, they are capable of assembling them in goodly numbers under the proper conditions. The implications of this finding are discussed.

REFERENCES

1. Prose, P. H.: Pathologic changes in eczema, in Conference on infantile eczema, (Holt, L. E. Jr., editor). J. Pediat., *66:* 178, 1965.
2. Gomori, G.: Microscopic Histochemistry; Principles and Practice, p. 193. Chicago, The University of Chicago Press, 1952.
3. Matoltsy, A. G. and Parakkal, P. F.: Membrane coating granules of keratinizing epithelia. J. Cell Biol., *24:* 297, 1965.
4. de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Applemans, F.: Tissue fractionation studies. VI Intra cellular distribution patterns of enzymes in rat liver tissue. Biochem. J., *60:* 604, 1955.
5. Gordon, G. B., Miller, L. R. and Bensch, K. G.: Studies on the intracellular digestive process in mammalian tissue culture cells. J. Cell Biol., *25:* 41, 1965.
6. Novikoff, A. B. and Essner, E.: The liver cell. Amer. J. Med., *29:* 102, 1960.
7. Ashford, T. P. and Porter, K. R.: Cytoplasmic components of hepatic cell lysosomes. J. Cell Biol., *12:* 198, 1962.

8. Novikoff, A. B. and Essner, E.: Cytolysosomes and mitochondrial degeneration. J. Cell Biol., *15:* 140, 1962.
9. Novikoff, A. B., Essner, E., Goldfischer, S. and Heus, M.: Nucleotidephosphatase activities of cytomembranes, in The Interpretation of Ultrastructure, p. 149, (Harris, R. J. C., editor), New York, Academic Press, 1962.
10. Eisen, A. Z., Arndt, K. A. and Clark, W. A.: The ultrastructural localization of acid phosphatase in human epidermis. J. Invest. Derm., *43:* 319, 1964.
11. Breathnach, A. S.: Observations on cytoplasmic organelles in Lagerhans cells of human epidermis. J. Anat., *98:* 265, 1964.
12. Mishima, Y.: Macromolecular changes in pigmentary disorders. Arch. Derm. (Chicago), *91:* 519, 1965.
13. Diengdoh, J. V.: The demonstration of lysosomes in mouse skin. Quart. J. Micr. Sci., *105:* 73, 1964.
14. Goldfischer, S., Essner, E. and Novikoff, A. B.: The localization of phosphatase activities at the level of ultrastructure. J. Histochem. Cytochem., *12:* 72, 1964.
15. Weissmann, G. and Thomas, L.: Studies on lysosomes. II The effect of cortisone on the release of acid hydrolases from a large granule fraction of rabbit liver induced by an excess of vitamin A. J. Clin. Invest., *42:* 661, 1963.

DISCUSSION

DR. WALTER F. LEVER, Boston, Mass.: It is most interesting that you have found lysosomes in the upper layers of the epidermis; but, I wonder whether the lysosomes actually were present within keratinocytes. Could they have been present within the melanocytes? We can find melanocytes in the upper layers of a rapidly proliferating epidermis, for instance in psoriasis and atopic dermatitis. I thought I saw a close association of the lysosomes with melanin granules in your electron microscopic pictures. I would like to ask you whether the three patients in whom the electron microscopic pictures were obtained were Negroes; because in Negroes one finds not only greater amounts of melanin in the epidermis than in white patients, but also greater amounts in the upper layers of the epidermis when the epidermis proliferates rapidly.

DR. ROBERT OLSON, Oklahoma City, Oklahoma: At Oklahoma we have recently found lysosomes in normal in addition to injured skin. We found this not only by acid phosphatase but also by showing DNase membrane limited structures. These are present in a great majority of basal cells. In our specimens taken from the antero-lateral aspect of the thigh, they were most numerous within the basal cells and did not persist into the normal stratum corneum.

DR. Y. MISCHIMA (Detroit, Michigan): I enjoyed your careful and important presentation very much. As I described previously (J. Cell Biol., *23:* 122A, 1964., Arch. Derm., *91:* 519–557, 1965), melanin phagocytosing lysosomes can occur in human keratinocytes which under the electron microscope are easily identified by their distinct tonofilament and desmosomes. We have also found that nevus spilus and Frain-Bell nevus (Trans. St. John's Hosp. Derm. Soc., *39:* 51, 1957) is due to an abnormally excessive formation of these melanin phagocytosing lysosomes in keratinocytes.

Correspondingly dermal melanophages also contain acid phosphatase rich melanin phagocytosing lysosomes as I will discuss tomorrow.

Would you comment on whether you have observed non-lysosomal acid phosphatase activity in human epidermis?

DR. PHILIP H. PROSE (in closing): In answer to Dr. Lever's question, these cells, in my own opinion, are keratinizing cells. They have within them tonofilaments and some of these were situated nowhere near melanocytes. The patients from whom biopsy specimens were taken were both Negroes and Caucasians. I thank Dr. Mishima for his comment. He pointed out that melanin granules can be degraded within keratinizing cells. As for non-specific acid phosphatase, we had no trouble with it when the specimens were incubated for a short period of time. However, we did not use an acid rinse for fear that we would remove acid phosphatase reaction products that were present.